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Estradiol does not influence strategy choice but place strategy choice is associated with increased cell proliferation in the hippocampus of female rats

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ABSTRACT

Adult neurogenesis occurs in the hippocampus of most mammals. While the function of adult hippocampal neurogenesis is not known, there is a relationship between neurogenesis and hippocampus-dependent learning and memory. Ovarian hormones can influence learning and memory and strategy choice. In competitive memory tasks, higher levels of estradiol shift female rats towards the use of the place strategy. Previous studies using a cue-competition paradigm find that 36% of male rats will use a hippocampus-dependent place strategy and place strategy users had lower levels of cell proliferation in the hippocampus. Here, we used the same paradigm to test whether endogenous or exogenous ovarian hormones influence strategy choice in the cue-competition paradigm and whether cell proliferation was related to strategy choice. We tested ovariectomized estradiol-treated (10 μg of estradiol benzoate) or sham-operated female rats on alternating blocks of hippocampus-dependent and hippocampus-independent versions of the Morris water task. Rats were then given a probe session with the platform visible and in a novel location. Preferred strategy was classified as place strategy (hippocampus-dependent) if they swam to the old platform location or cue strategy (hippocampus-independent) if they swam to the visible platform. All groups showed a preference for the cue strategy. However, proestrous rats were more likely to be place strategy users than rats not in proestrus. Female place strategy users had increased cell proliferation in the dentate gyrus compared to cue strategy users. Our study suggests that 78% of female rats chose the cue strategy instead of the place strategy. In summary the present results suggest that estradiol does not shift strategy use in this paradigm and that cell proliferation is related to strategy use with greater cell proliferation seen in place strategy users in female rats.

The hippocampus is an important brain structure for certain types of learning and memory (Broadbent et al., 2004; Scoville and Milner, 1957). However other brain areas, such as the dorsal striatum, are also important for some forms of learning and memory and represent complementary mnemonic systems (McDonald and White, 1993). McDonald and White (1993) provided evidence for the existence of multiple memory systems by showing a triple dissociation by damaging the hippocampus, amygdala and striatum individually and comparing the performance before and after lesions on different versions of the radial arm maze. Consistent with other studies, they found that the hippocampus is required for the spatial version of the radial arm maze. However lesions of the dorsal striatum resulted in impaired performance on the win-stay task. Therefore, depending on the type of information available different neural systems are responsible for the information processing.

Estradiol levels can influence hippocampus-, and striatum-dependent learning in female rats in a dose-dependent manner (Galea et al., 2001; Holmes et al., 2002; Sinopoli et al., 2006). High levels of estradiol impaired performance on the working/reference memory version of the radial arm maze, and win-stay task, the same tasks that were used by McDonald and White (1993). These data suggest that estradiol can differentially influence hippocampus- and striatum-based learning in female rats.

In many tasks where multiple memory systems can be applied, the ratio of the contribution of each neural system determines what kind of strategy is used. McDonald and White (1994) utilized the cue-competition version of the Morris water task that can be solved by the use of either the dorsal striatum or the hippocampus. McDonald and White trained the rats to swim to a visible platform, which remained at the same position throughout training. After three days of testing, the visible platform was replaced with a hidden platform in the same location. This training was repeated three times. On the final day, the visible platform was placed in the opposite quadrant of the pool. Approximately 50% of male rats were place responders, swimming to the previously trained spatial location, while the others were cue responders, swimming directly to the visible platform. Rats with

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damage to the dorsal striatum preferentially swam to the old spatial position of the platform and were considered as 'place responders'. In contrast rats with damage to the hippocampus (fornix) swam directly to the visible platform and were considered 'cue responders'.

There is evidence that ovarian hormones, including estradiol, can influence strategy choice in different types of cue-competition tasks (Daniel and Lee, 2004; Korol et al., 2004; Korol and Kolo, 2002). Korol et al. (2004) used a task which can be solved by using either the striatum or the hippocampus. They found that rats in proestrus or rats that received estradiol chose the hippocampus-dependent place strategy significantly more often than the hippocampus-independent response strategy (about 70:30) (Korol et al., 2004; Korol and Kolo, 2002). However, the majority of rats (70%) in estrus preferred the response strategy to the place strategy. Thus, as suggested by Korol et al. (2004), ovarian hormones can alter the ratio of contribution of the different memory systems which could result in a shift of strategy choice.

As mentioned earlier, integrity of the hippocampus is important for determining the proportion of place strategy users and thus these preceding studies suggest that the efficiency of the hippocampus (which controls place learning and place strategy use) and striatum (which controls response learning and cue strategy use) is affected by estradiol. Certainly estradiol influences long-term potentiation in the hippocampus and dopamine release in the striatum (Córdoba Montoya and Carrer, 1997; Pasqualini et al., 1995; Smith and McMahon, 2005; Van Hartesveldt and Joyce, 1986). Interestingly, cell proliferation in the hippocampus is influenced by estradiol (for review see Galea, 2008). Studies from our laboratory have shown that estradiol administration initially increases the rate of cell proliferation (within 4 h) but subsequently decreases the rate of cell proliferation (24–48 h) (Ormerod et al., 2003; Ormerod and Galea, 2001), while repeated administration on estradiol may increase cell proliferation in the hippocampus of adult female rodents (Barker and Galea, 2008). Thus the effect of estradiol on cell proliferation depends on time and duration of exposure. Recently we found that male rats that preferentially use a place strategy had lower levels of cell proliferation in the hippocampus (Epp and Galea, 2009), suggesting that higher rates of cell proliferation may be associated with a less efficient hippocampus, a finding supported by computer modelling (Butz et al, 2006).

In this study we sought to determine whether estradiol levels shifted strategy choice in the cue-competition version of the Morris water task and whether the same relationship between strategy choice and cell proliferation in the hippocampus existed in female rats. We used the paradigm used by Epp and Galea (2009) and used intact or ovariectomized rats (which were treated with either estradiol benzoate or vehicle). Based on the studies previously described, we expected estradiol-treated and proestrous rats to have a preference for a spatial strategy. In addition, based on work in males, we expected spatial responders to have decreased levels of cell proliferation and that approximately 40% of females would prefer a spatial strategy (Epp and Galea, 2009).

Methods

Animals

Seventy-one female Long–Evans rats purchased from Charles River Laboratories (Quebec, Canada) were used. Rats were initially double-housed in standard opaque cages and were single-housed after surgery. The rats had *ad libitum* access to water and lab chow (Purina LabDiet) and were kept on a 12/12 h light/dark cycle with lights turned on at 7 am. Rats weighed approximately 230–270 g at the beginning of the experiment. All animals were cared for in accordance with the ethical guidelines of the Canada Council for Animal Care and were approved by the local animal care committee at the University of British Columbia.

Ovariectomy

After a habituation phase of seven days, rats were either bilaterally ovariectomized (OVX; n = 32) or sham-ovariectomized (n = 18). Sham surgery included bilateral incisions but the ovaries were not excised. Rats were anesthetized using an initial flow rate of 5% isofluorane (Boxter Corp., Mississauga, ON, Canada) and 3% during surgery. Five mg/kg Anafen (Merial Canada Inc, Baie-d'Urfe, QC, Canada) was administered subcutaneously (s.c.) before surgery. To prevent dehydration, 5 ml Lactated Ringer Solution (Braun Medical Inc, Scarborough, ON, Canada) was injected s.c. After a recovery phase of six days, daily lavage samples were taken for all animals and injections of hormone or vehicle were initiated for all rats.

Vaginal lavage

A few drops of saline were released from a glass pipette into the rats' vagina and sucked back into the pipette. The saline, which now contained vaginal cells, was transferred onto microscope slides. When the samples dried, they were stained with Cresyl Violet (Sigma) and analyzed using a 20× objective. Proestrous stage was determined when 70% of the cells were nucleated epithelial cells. Daily lavage samples were taken from all rats immediately after injections (see below) and in order to keep the experience the same for all subjects.

Injections

Rats were handled for four days prior to any injections. On day one, starting at 9 am, OVX rats received a s.c. injection of either estradiol benzoate (Sigma) (10 μg/0.1 ml sesame oil) or vehicle (0.1 ml sesame oil) for 10 consecutive days including the probe trial. Immediately after the injections, lavage samples were taken. Two hours after injections, the animals were tested in the Morris water task.

Apparatus

The pool measured 180 cm in diameter and was filled 30 cm deep with water. The water had a temperature of about 21 °C and was rendered opaque using white nontoxic paint. Visual cues were placed on the walls surrounding the pool. Above the center of the pool, a camera was installed and connected to a computer running ANY-MAZE (Stoelting, Wood Dale, IL, USA). Latency, distance and average speed to reach the platform were analyzed by this video tracking system.

Procedure

A platform 10 cm in diameter was placed in the northeast quadrant of the pool, where it stayed throughout the whole experiment except for the probe trials. Each rat had a total of four trials per day. For each trial, rats were released into the pool facing the wall of the pool from one of four positions which were chosen in a pseudo-random order. A trial stopped when the rat reached the platform or when 60 s elapsed. If the animal was not able to find the platform within this time, the investigator led the rat to the platform. Rats were given 10 s on the platform prior to being removed and placed back into the holding cage. On the first two days, the platform extended 2 cm above the surface (visible trial). On the third day, the platform was submerged 2 cm below the water surface (hidden trial). The testing procedure started at 11 am and was repeated for nine days so that the rats received a total of six days of visible platform training and three days of hidden platform training. On day 10, two probe trials took place each a maximum of 60 s in duration. The visible platform was moved into the middle of the southwest quadrant of the pool. Rats were released from two different spots between the new and the old location of the platform and the trial was terminated once the rat reached the visible platform or once 60 s elapsed. Rats were